Sortase A

Week 5

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 PCR of *S.aureus* genome to isolate truncated Sortase A gene

Responsible

Aman Mebrahtu, Bethel Tesfai Embaie and Reskandi Rudjito

Protocols used

Q5® High-Fidelity 2X Master Mix

Modifications and comments to protocols

No Modifications were made to the protocol. Two samples with one replicate each was prepared for PCR,
resulting in a total number of four samples. The total volume of each reaction was set to 25 μl. Annealing temperature was set to 52 °.
$Forwardprimer : SortA_F$ $Reversedprimer : SortA_R$

Experimental Data

Table 1: Samples DNA 1 E1 and sample DNA 2 E1, containing extracted S.aureus genome, were used for isolation of the Sortase A gene. Duplicates were made for both samples.

Sample	Concentration [ng/ul]
DNA 1 E1	218.4
DNA 1 E2	55
DNA 2 E1	149.4
DNA 2 E2	86.1

Sample Calculation

Table 2: PCR Experimental set-up

Component	25 μl Reaction	Final Concentration
10 μM Forward Primer	1.25	0.5 μM
10 μM Reverse Primer	1.25	0.5 μM
Template DNA	1	DNA 1: 218.4 ng, DNA 2: 149.4 ng
Q5 High-Fidelity 2x Master Mix	12.5	0.02 U/μl
Water	14.75	

Results and Conclusions

The expected size of the Sortase gene at 491 bp is observed for all four samples in the gel (Figure 1). Further, no other unspecific bands are observed on the gel and the negative control shows no sign of amplification. In conclusion, isolation of truncated Sortase A gene was successful.

Table 3: The concentration of the samples after PCR, measured with NanoDrop at 280 nm.

Sample	Concentration [ng/ul]
DNA 1.1	34.2
DNA 1.2	35.4
DNA 2.1	39.7
DNA 2.1	41.8

Discussion and Troubleshooting

Following the successful isolation of the Sortase A gene, subsequent steps will focus on fusing the gene with a the GB1 domain, a solubility tag, by either Gibson assembly or Overlap extension PCR.

References

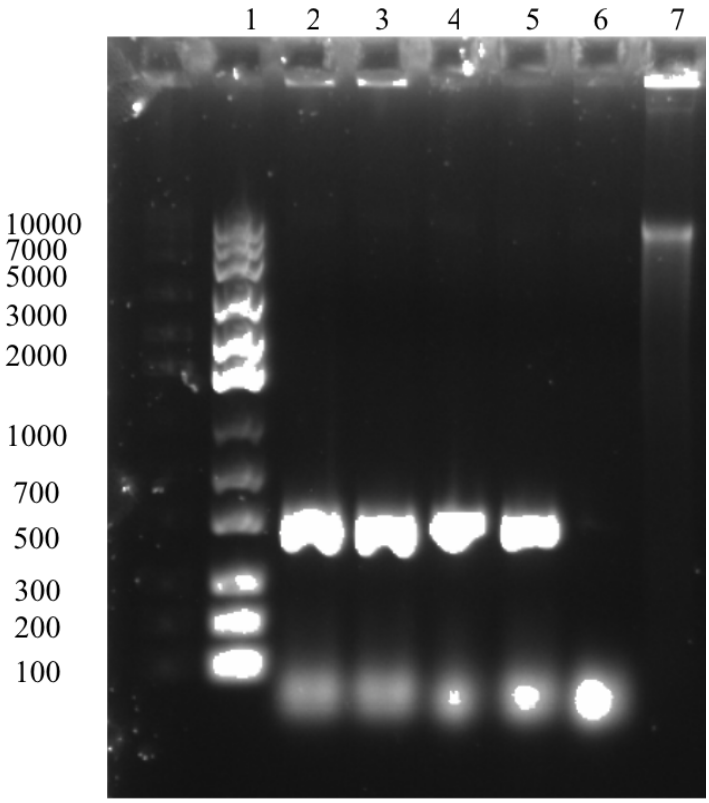

Figure 1: Result from gel electrophoresis in 1 % agarose, at 110 V. (1) DNA Ladder (2) DNA 1.1 (3) DNA 1.2 (4) DNA 2.1 (5) DNA 2.2 (6) Negative control (7) S.aureus genome

2 Gibson assembly of GB1 Domain and Sortase A

Responsible

Aman Mebrahtu, Bethel Tesfai Embaie and Reskandi Rudjito

Protocols used

Gibson assembly

Modifications to protocols

No vector was added to the reaction mix.

Experimental Data

Samples used for the assembly from **Experiment 1** were *DNA 1.1* and *DNA 2.1*
Expected size of assembled product:

Table 4: Sizes of the different components and the expected size of assembled product.

Component	Size [bp]
GB1	191
Sortase A	491
GB1-Sortase A (GS)	662

Sample Calculation

The desired amount DNA in pmoles for each sample was 0.1 pmoles according to the protocol. Calculation of pmoles in each Sortase A DNA sample was made with the following assumptions and equations:
Moles dsDNA = mass of dsDNA (g)/molecular weight of dsDNA (g/mol) molecular weight of dsDNA = (number of base pairs of dsDNA x average molecular weight of a base pair) + 36.04 g/mol average molecular weight of a base pair = 617.96 g/mol, excluding the water molecule removed during polymerization. The 36.04 g/mol accounts for the 2 -OH and 2 -H added back to the ends. Bases are assumed to be unmodified (NEB REFERENCE TO CALCULATOR).
The following moles were calculated for the two samples used:

Table 5: Calculated pmoles in the two samples of Sortase A DNA used for Gibson Assembly

Sample	Concentration [ng/μl]
DNA 1.1	0.113
DNA 1.2	0.131

The following experimental set-ups for Gibson assembly were performed

Table 6: Trial 1.

Trial 1 - Set Up	Components	Description
Gibson DNA 1.1 + GB1	Gibson Mastermix + DNA 1.1 + GB1	Dilluted DNA
Gibson DNA 2.1 + GB1	Gibson Mastermix + DNA 1.2 + GB1	Dilluted DNA
Negative	GB1 + DNA 1.1	Dilluted DNA
Sortase A	Sortase A	-
GB1	GB1	-

Table 7: Trial 2.

Trial 2 - Set Up	Components	Description
Gibson DNA 1.1 + GB1 (2 ng/μl)	Gibson Mastermix + DNA 1.1 + GB1	Undilluted DNA
Gibson DNA 2.1 + GB1 (3 ng/μl)	Gibson Mastermix + DNA 1.2 + GB1	Undilluted DNA
Negative	GB1 + DNA 1.1	Undilluted DNA
Sortase A	Sortase A	Undilluted DNA
GB1	GB1	Undilluted DNA

Results and Conclusions

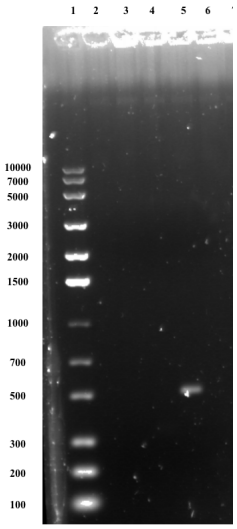

Figure 2: Result of from gel electrophoresis in 1 % agarose, at 110 V. (1) DNA Ladder (2) DNA 1.1 + GB1 (3) DNA 2.1 + GB2 (4) Negative control (5) Sortase A (6) GB1

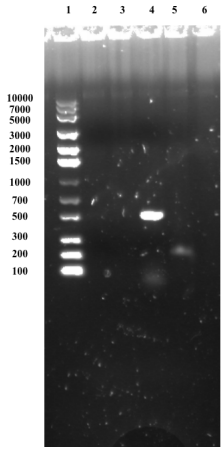

Figure 3: Result of from gel electrophoresis in 1 % agarose, at 110 V. (1) DNA Ladder (2) DNA 1.1 + GB1 (3) DNA 2.1 + GB2 (4) Sortase A (5) GB1 (6) Negative control

Discussion and Troubleshooting

The expected size of 622 bp could not be observed in none of the trials made for Gibson Assembly of the Sortase A gene and GB1 domain gene. A plausible explanation, is that no vector was added to the reaction mix, for which the protocol was designed for. A second strategy will be tested to fuse the two sequences together by overlap extension PCR, using a forward primer designed to anneal upstream the Sortase A sequence with an 20 bp overlap with the exact same sequence as the last 20 bp of the GB1 domain gene.

References

3 PCR of Sortase A gene

Responsible

Bethel Tesfai Embaie and Reskandi Rudjito

Protocols used

Q5 High Fidelity 2x Master Mix

Modifications and comments to protocols

No modifications were made to the protocol, Annealing temperature was set to 52 °C
Total reaction volume: 20 μl

Experimental Data

Table 8: Samples DNA 1.1 and sample DNA 2.1 containing extracted S.aureus genome, were used for isolation of the Sortase A gene. Duplicates were made for both samples.

Sample	Concentration [ng/μl]
DNA 1.1	218.4
DNA 2.1	149.4

Table 9: PCR Experimental Set-up.

Component	25 μl Reaction	Final Concentration
5X Q5 Reaction Buffer	5	1X
10 mM dNTPs	0.5	200 μM
10 μM Forward Primer	1.25	0.5 μM
10 μM Reverse Primer	1.25	0.5 μM
Template DNA	2	1 ng
Q5 High-Fidelity DNA Polymerase	0.25	0.02 U/μl
Water	14.75	

Results and Conclusions

Table 10: The concentration of the samples after PCR, measured with NanoDrop at 280 nm.

Sample	Concentration [ng/ul]
SortA 1	93.5
SortA 2	105

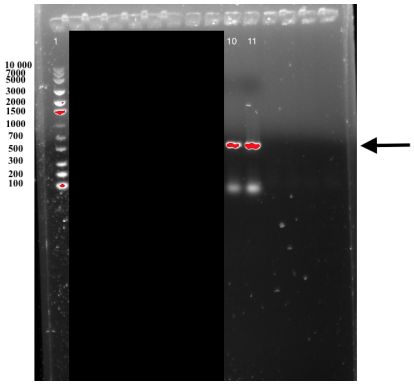

Figure 4: Gel electrophoresis results in 1 % agarose, at 110 V. (1) DNA Ladder (10) DNA 1.1 (Labeled SortA 1) (11) DNA 2.1 (Labeled SortA 2). A band for both samples of the expected size around 500 bp can be observed.

Discussion and Troubleshooting

Following PCR of the Sortase A gene, a new strategy will be tested to fuse it with the GB1 Domain solubility tag through the means of overlap extension PCR.

References